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Molecular Basis of Asymptomatic β-Thalassemia Major in an African American Individual

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The β-thalassemia syndromes are a heterogeneous group of genetic disorders characterized by reduced or absent expression of the β-globin gene. To date, over 300 β-thalassemia alleles have been characterized in or around the β-globin region. Thalassemia major is severe anemia necessitating chronic blood transfusions, splenectomy, iron chelation therapy, and bone marrow transplantation. Usually thalassemia major results from homozygosity or compound heterozygosity for severe β^{O}- and/or β^{+}-thalassemia mutations. Thalassemia intermedia is a clinical diagnosis that describes a symptomatic but less severe condition than β-thalassemia major. β-thalassemia intermedia may arise from several different combinations of α- and/or β-thalassemia mutations. Heterozygous β-thalassemia is typically characterized by a mild microcytic hypochromic anemia without any significant clinical implications. In this report, we describe a 63-year-old African American woman with asymptomatic homozygous β-thalassemia, who seems to carry 2 copies of the −29 mutation in the promoter region of the β-globin gene. Her elevated hemoglobin F level of 83% was associated with heterozygosity for the Xmn I polymorphism upstream of the $^{G}\gamma$-globin gene. Southern blot analysis at the α-globin locus did not show any deletion that would account for the mildness of her phenotype. Therefore, homozygosity for the −29 mutation along with the Xmn I polymorphism appears to confer an extremely mild β-thalassemia phenotype. This observation has important implications in the prenatal diagnosis and genetic counseling of families segregating this type of genetic defect. Am. J. Med. Genet. 69:196–199, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: β-thalassemia major; Cooley's anemia; reverse dot blot hybridization; PCR sequencing

INTRODUCTION

The β-thalassemias, a group of disorders caused by reduced hemoglobin synthesis, are genetically heterogeneous and clinically variable [Weatherall and Clegg, 1981; Weatherall et al., 1989; Bunn and Forget, 1986]. These autosomal recessive disorders, characterized by absent (β^{O}) or reduced (β^{+}) production of the β-globin chains of the hemoglobin tetramer, result in imbalanced globin chain synthesis which is the major determinant of the clinical course of the disease [Weatherall and Clegg, 1981; Weatherall et al., 1989; Bunn and Forget, 1986]. At the molecular level, most of these defects are subtle mutations within the β-globin gene or its immediate flanking regions resulting either in non-functional truncated proteins, or absent or reduced β-globin polypeptide synthesis [Huisman, 1992; Kazazian, 1990]. We report an African American individual homozygous for a β-globin gene promoter mutation and heterozygous for a polymorphism previously shown to be associated with high levels of hemoglobin F. The interesting aspect of this individual is her normal clinical picture and the extremely mild course of her thalassemia intermedia.

MATERIALS AND METHODS

Hematologic Analysis

Hemoglobin (Hb), hematocrit (Hct), reticulocyte count, and RBC indices were determined by routine laboratory methods. Hb electrophoresis was performed on cellulose acetate (pH 8.6) & citrate agar (pH 6.2) and was confirmed by thin layer isoelectric focusing. The

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percentage of Hb A_2 was quantitated by microcolumn chromatography as described by Huisman et al. [1975] and Hb F was determined by alkali denaturation [Betke et al., 1959]. The percentage of RBCs in peripheral blood that contains Hb F was determined by the Kleihauer-Betke acid elution technique. In vitro synthesis of globin chain was determined after incubating peripheral blood reticulocytes with uniformly labeled ^{14}C-leucine for two hours at 37°C and after separating the labeled globin chains on carboxymethyl cellulose chromatography in 8M urea [Clegg et al., 1966].

DNA Analysis

DNA was extracted from peripheral blood leukocytes and analyzed by Southern blotting as previously described [Poncz et al., 1982]. The number of α-globin genes was determined by hybridizing Bam H1- and Bgl II-digested DNA with α-gene probe [Ballas et al., 1987]. Haplotype analysis using 9 restriction fragment length polymorphisms (RFLP) in the β-globin gene cluster were identified using appropriate restriction enzymes and probes as previously described [Ballas et al., 1987; Antonarakis et al., 1984; Ballas et al., 1991].

DNA Amplification and Reverse Dot Blot Hybridization

Allele specific oligonucleotide (ASO) hybridization of amplified PCR products was performed as described previously [Cai et al., 1994; Chehab and Wall, 1992; Maggio et al., 1993]. Two 50 μl amplification reactions were set up for each DNA sample to be typed. The first reaction included 3 primers and generated two PCR products: 1503 bp (from primers China 1/China 4) and 162 bp (from primers China 1/PC05). The second reaction amplified a 597-bp product with the primers PC03, and PC06. Each amplification reaction was performed in a standard buffer containing 1.5 mM MgC12, 100 μM dNTPs, and 10 μM biotin-11-dUTP for 35 cycles (94°C, 60 seconds, 55°C, 30 seconds, 72°C, 30 seconds). The strips were prehybridized individually in 5 mL of 2XSSC (1XSSC is 0.15 mol/L sodium chloride, 0.015 mol/L sodium citrate), 0.1% sodium dodecylsulfate (SDS) at 45°C for 30 minutes. Ten microliters from each amplification reaction were denatured by boiling for 5 minutes, added both to a membrane strip containing probes specific for the Black population, and hybridized for 45 minutes. The membranes were then washed in 2XSSC at 45°C for 10 minutes, then transferred to 20 mL of 2XSSC containing 5 U of streptavidin horseradish peroxidase (HRP) conjugates (Boehringer-Mannheim, Germany) for 30 minutes at room temperature. The color detection was essentially performed as described in the Boehringer-Mannheim Genius Kit and the color detection substrate was tetramethylbenzidine (TMB).

RESULTS

Case History

The patient studied was a 63-year old African American woman who was referred because of essential thrombocytosis with a platelet count of 517–767 × 10^6/μl.

TABLE I. Summary of Hematological Data

Parameter	Result
Hb, g/dl	11.3
Hct, %	35.8
MCV, fl	66
MCH, pg	20.9
MCHC, g/dl	31.6
Reticulocytes, %	3.0
Hb A, %	14.5
Hb A2, %	2.5
Hb F, %	83
Gγ/Aγ	1.3
α/βSynthetic ratio	3.03
α-Genotype	αα/αα
β-Haplotype	3/12 (Senegal/Car variant)

Table I lists results of routine and special hematological studies performed on her peripheral blood and Table II illustrates her β-haplotype. There was no evidence of iron deficiency anemia and there was no past history or family history of anemia or hemoglobinopathy. The Kleihauer-Betke acid elution technique showed that the distribution of Hb F was pancellular in peripheral blood in that 100% of red cells contained detectable Hb F.

Mutation Detection

Figure 1 shows a strip that has been hybridized with PCR-amplified DNA from the proposita. The DNA sequence at each mutation site is represented by a normal and a mutant oligonucleotide probe that differ by a single base substitution. The presence of a particular sequence can be detected by the appearance of a dot on the membrane. Amplified DNA from the proposita hybridized to the −29 mutant probe but not to its corresponding normal sequence thus indicating homozygosity for the A to G transition at position −29 relative to the cap site of the β-globin gene.

Haplotype Analysis

Analysis of nine common restriction site polymorphisms in the β-globin gene cluster showed that the proposita was double heterozygote for haplotypes 3 and 12 (Table II). The combination of these two haplotypes results in heterozygosity for the Xmn I polymorphism, upstream of the $^{G}\gamma$ globin gene.

DISCUSSION

The striking aspect about this patient is the unusually asymptomatic phenotype of her β-thalassemia despite the fact that she has some of the classical findings of thalassemia such as microcytic, hypochromic RBC indices and decreased synthesis of the β-globin chains. Another unusual characteristic about this patient is her 80% level of Hb F and her lack of need for transfusions. It is possible that the mildness of her phenotype might have been contributed by her gender and heterozygosity for the Senegalese haplotype [Gilman, 1988; Miyoshi et al., 1988; Nagel, 1991].

Although homozygosity for the −29 deletion is most likely in this patient other molecular mechanisms may explain our findings. These include compound hetero-

TABLE II. Reported Haplotypes of β-thal Chromosomes Containing the −29 Mutations*

Haplotype	ϵ	Xmn 1	GγAγ	wβ	β Ava II	β Hpa I	β Bam H1	Reference
3			+ −	+ +	+	+	+	Tuan et al. [1980]
Atypical			− −	+ +	+	+	+	Tuan et al. [1980]
24	+	−	− −	− −	+	+	−	Fritsch et al. [1979]
3	−	+	+ −	+ +	+	+	+	Gozales-Redondo et al. [1988]
Atypical	−	−	− −	− +	+	+	+	Gozales-Redondo et al. [1988]
3	−	+	+ −	+ +	+	+	+	This report
12	−	−	− −	− −	+	+	+	This report

*Haplotype assignment was as described by Antonarakis et al. [1984].

zygosity for the −29 β-thalassemia mutation and some β-globin gene deletions several of which have been described in the African American population. These include the following: 1) Hereditary persistence of fetal hemoglobin (HPFH) types 1 and 2 [Tuan et al., 1980; Fritsch et al., 1979]; 2) A 12 Kb deletion that encompasses the β-globin gene [Anagnou et al., 1985]; and 3) $\delta\beta^{O}$-thalassemia or $A\gamma\delta\beta^{O}$-thalassemia [Henthorn et al., 1985]. No attempt was made to rule out these possibilities in this patient since this will not change the genotypic diagnosis of thalassemia major (β^{+}/β^{+} or β^{+}/β^{O}). The fact that Hb F was present in 100% of red cells in peripheral blood makes the co-existence of $\delta\beta^{O}$ and $A\gamma\delta\beta^{O}$-thalassemia unlikely.

The −29 (A → G) β-thalassemia mutation in the promoter region of the β-globin gene is prevalent among African Americans and results in a mild reduction of β-globin mRNA [Antonarakis et al., 1984] The proposita in this report has the mildest homozygous β-thalassemia among the other patients homozygous for the −29 mutations that were reported [Antonarakis et al., 1984; Huang et al., 1986]. The two African American sibs 21 and 16 years old, reported by Antonarakis et al. [1984], were also mildly affected and did not require blood transfusion. Their Hb level was 9–10 g/dl, their Hb F levels were 50%, and they had no deletion of α-genes. A Chinese man [Huang et al., 1986] was 21 years old when reported, had splenectomy at age 11 years, required blood transfusion 4–5 times per year before splenectomy and 1–2 times post splenectomy, and had Hb F level 45%. The ages of the 5 African Americans reported by Gonzalez-Redondo et al. [1988] varied between 8 and 44 years, their hemoglobin between 7.7 and 11.4 g/dl, their Hb F between 11.9 and 68%, and their clinical course was mild. As shown in Table II, most of the African American patients were homozygous for the Senegalese haplotype whereas the Chinese patient had the Asian haplotype (+? −− −− ++−). It is therefore apparent that the −29 mutation on the Asian genetic background is more penetrant than on the African American genetic background, thus, demonstrating the effect and existence of modifier genes that affect the clinical course of β-thalassemia. Our patient was 63 years old, was never transfused, and was completely asymptomatic. To the best of our knowledge she has the mildest homozygous β-thalas-

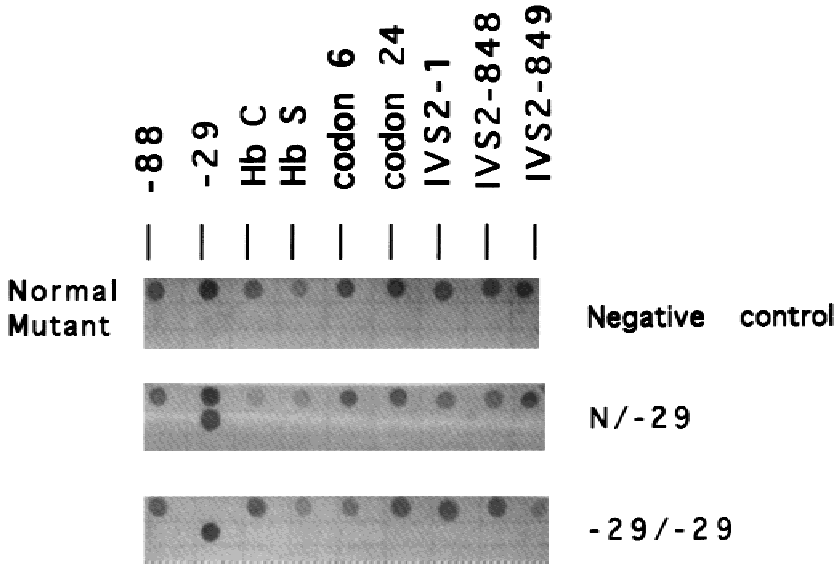

Fig. 1. Detection of β-thalassemia mutations, Hb S and Hb C by PCR and reverse dot blot hybridization followed by color development. Each strip represents a hybridization from a single individual whose genotype is shown next to the strip. The normal and mutant probes for each mutation are dotted, respectively, on the upper and bottom rows of each strip and in the same order for all strips. The upper strip shows a signal with all the normal probes but without any mutation probe. The middle strip shows heterozygosity for the −29 mutation. The lower strip shows the patient described in this paper and indicates that she is homozygous for the −29 mutation.

semia major reported in African Americans and perhaps in other ethnic groups. The mildness of her phenotype could be attributed at least in part to the nature of the −29 mutation and also to the presence, on one chromosome, of the Xmn I site which was shown to be associated with high levels of Hb F. Other yet uncharacterized factors that may influence such an elevated level of Hb F could arise from other Hb F determinants such as the one mapped to the X-chromosome [Miyoshi et al., 1988]. Overall, the mildness of the thalassemia intermedia in this case is not fully accountable by only the nature of the −29 mutation, but requires also other genetic factors that appear to be present in the African American population, thus providing a protective effect against a severe clinical course of β-thalassemia.

The −29 mutation and its association with the Xmn I polymorphism on African American genetic background has important implications in genetic counseling and prenatal diagnosis. Although genotype-phenotype correlations in thalassemia intermedia are difficult to predict in a prenatal diagnosis setting, it is very likely that a mild picture in one individual is likely to be maintained within his/her family, provided that the same determinants are inherited. Therefore, genetic counseling should provide the delicate understanding of these situations such that well informed parents can reach a wise decision.

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